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Pterocarpus marsupium

Indian kino tree (*Pterocarpus marsupium* Roxb.) is a keystone deciduous tree generally known as Indian kino tree or Malabar kino (English), Vijayasar (Hindi) and Biyo (Gujrati). It is an important multipurpose medicinal tree belonging to the family Fabaceae.

Among the diverse flora of the Indian subcontinent, Indian kino tree occupies a critical position on the IUCN Red List, currently categorized as a "Near Threatened" species (IUCN, 2017). The genus *Pterocarpus* consists several tropical tree species distributed across Asia and Africa. Indian kino tree is native to the Indian subcontinent and is widely distributed in the tropical and subtropical regions of India, Sri Lanka and Nepal. In India the species is generally found in the states of Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Kerala and parts of the Western Ghats.

It usually grows in dry deciduous and mixed forests at elevations ranging from about 200 to 1200 meters above sea level. Beyond its high-density timber, the species

is globally recognized for its unique pharmacological profile, specifically the bioactive C-glycosides found in its heartwood and "Kino" exudate (Barmukh and Nikam, 2008).

Indian kino tree is a large deciduous tree that can reach upto height of about 20-30 meters with a broad spreading crown. The tree produces yellow papilionaceous flowers arranged in terminal or axillary panicles usually during the late winter or early summer months. Pollination is mainly entomophilous involving insect pollinators. The fruit is a flat, circular and indehiscent winged pod containing a single seed. The seeds are 1 to 2 cm and hard (Khan, 2015).

The natural regeneration through seeds is difficult due to the physical limitation to moisture diffusion caused by hard and thick seed coat which are responsible for seed dormancy (Das and Chatarjee, 1993), the seeds of Fabaceae family have impermiable seed coat (Al-Menaie *et al*., 2010). The seeds are enclosed in winged pods (samaras), are often subjected to high rates of environmental stressors and inherent physiological dormancy (Patel and Patel, 2016). However, the most significant barrier to conservation is the rapid loss of seed vigor shortly after dispersal, which complicates the maintenance of a consistent supply of high-quality planting material (Behera *et al.*, 2026).

Natural seed ageing is a physiological process that leads to gradual loss of viability and vigour during storage. During ageing, several biochemical changes occur in the seed, such as reduction in enzyme activity and degradation of cellular components. These changes directly affect germination and seedling growth.

The trajectory of these aging processes is highly sensitive to external variables, specifically ambient temperature and relative humidity. Improper storage conditions can accelerate the biochemical leaching process, leading to the rapid exhaustion of the "planting value" of the seeds (Walters *et al*., 2010). Identifying the specific "storage fingerprints" of *P. marsupium* is essential for developing protocols that can extend the shelf-life of these seeds while maintaining their genetic potential (Ellis and Roberts, 1981).

Seed viability and vigor are critical factors influencing successful germination, seedling establishment and plantation development in forest tree species. In many tropical tree species, seed viability declines rapidly during storage due to physiological and biochemical changes within the seed tissues. Evaluation of seed viability through germination tests and physiological indicators helps in understanding seed quality and predicting field performance. In addition, biochemical constituents such as proteins,

sugars, phenolic compounds and antioxidant molecules play an important role in seed metabolism, germination and early seedling growth.

The species is well known for its rich phytochemical composition and medicinal significance. The heartwood is used in the treatments of kapha and pitta, inflammation, excessive urination, intestinal worms, rheumatoid arthritis haemorrhages, leprosy fractures, bruises, leukoderma, leprosy, skin diseases, elephantiasis, cough, erysipelas, pain in the rectum, diarrhoea, dysentery, rheumatoid arthritis, asthma, diabetes, bronchitis and greyness of hair (Maneesha *et al*., 2015). These compounds possess strong antioxidant, antidiabetic, anti-inflammatory and antimicrobial properties. Because of these biochemical constituents Indian kino tree has been widely used in traditional Ayurvedic medicine for the treatment of diabetes, skin diseases and other metabolic disorders.

Although Indian kino tree is an important medicinal tree species, limited information is available on the effect of natural aging, storage conditions and storage duration on seed quality and biochemical parameters. Hence, the present investigation was undertaken to study the effect of natural seed ageing and storage conditions on seed quality attributes and biochemical characteristics of Indian kino tree (*Pterocarpus marsupium* Roxb.).

The present study on Indian kino tree is expected to generate valuable scientific information with practical applications in seed science, forestry and medicinal plant utilization.

Evaluation of seed viability and vigor using standard physiological tests will provide essential insights into seed quality and storage behavior. The information generated will be useful for determining optimal storage conditions, maintaining seed longevity and ensuring better field establishment. Such findings can be effectively utilized by forest departments, seed technologists and researchers for the production and management of high-quality seeds.

The study will also generate useful insights into the effect of different storage conditions and storage durations on seed quality attributes. Understanding the appropriate storage environment and duration will help in maintaining seed viability for a longer period and reducing the losses caused by natural seed ageing. This will ultimately improve seed handling, storage practices and availability of quality seeds throughout the year.

Currently, the "Near Threatened" status of *P. marsupium* is exacerbated by a lack of reliable protocols for long-term seed storage, leading to significant germplasm wastage. By identifying the specific temperature and moisture thresholds that trigger natural aging, this study provides a "Technical Blueprint" for foresters and conservationists. Implementing these optimized storage conditions will allow for a consistent, year-round supply of high-quality seeds, ensuring that large-scale plantation programs are no longer restricted by the short natural viability window of the species.

In addition, the study will provide information on biochemical changes occurring in seeds during storage, which are closely associated with seed deterioration and loss of vigour. Understanding these changes will help in developing better strategies for seed quality management and long-term conservation.

Overall, the findings of the present study will provide a better understanding of the effect of natural seed ageing and storage conditions on seed quality attributes and biochemical characteristics of Indian kino tree (*Pterocarpus marsupium* Roxb). The information generated will be helpful in developing effective strategies for maintaining seed viability and vigour during storage, thereby improving seed quality management and conservation of this important medicinal species.

1. To assess the effect of natural ageing on seed quality attributes of Indian kino tree (*Pterocarpus marsupium* Roxb)
2. To study the effect of various storage conditions on seed quality attributes of Indian kino tree (*Pterocarpus marsupium* Roxb)
3. To study the effect of various storage durations on seed quality attributes of Indian kino tree (*Pterocarpus marsupium* Roxb)
4. To study the changes in biochemical parameters of seeds during storage in Indian kino tree (*Pterocarpus marsupium* Roxb)

The review of literature pertaining to the objectives of the present study in Indian kino tree is very scarce. Therefore, such review of other crops is reviewed and presented herewith.

Pterocarpus marsupium

Abdellaoui *et al.* (2013) evaluated the physiological and biochemical transitions occurring in *Periploca angustifolia* seeds to determine the metabolic drivers of long-

term senescence due to natural aging. The study specifically monitors the depletion of soluble sugars and polyphenol concentrations alongside the decline in radical scavenging activity as indicators of cellular aging. Furthermore, it seeks to quantify how the loss of antioxidant defenses leads to an accumulation of free radicals and subsequent membrane leakage, as measured by electrical conductivity. By identifying the specific moisture thresholds that distinguish after-ripening from terminal deterioration, the objective is to define the functional lifespan and optimal preservation markers for this species.

Kumar (2019) evaluated the physiological and biochemical decline in *Trigonella foenum-graecum* by comparing the effects of natural and accelerated aging across various genotypes. The study aims to quantify the reduction in germinability, vigour and enzymatic efficiency that occurs as storage duration progresses, while monitoring the corresponding increase in cellular membrane permeability. Additionally, the objective is to assess the restorative potential of seed priming agents, specifically GA_3 and thiram, in mitigating age-related damage. By analysing these parameters under both laboratory and field conditions, the study seeks to establish effective protocols for revitalizing aged seed lots and enhancing overall seedling establishment.

Singh *et al.* (2015) evaluated the natural aging process across different genotypes of fenugreek to identify the specific physiological and biochemical markers of long-term viability loss. By analysing seed lots stored for up to three years, the study tracks the progressive decline in seedling vigour and the corresponding increase in membrane permeability, as evidenced by elevated electrical conductivity. Furthermore, it leads to reduction in metabolic efficiency and germination potential to determine which genetic lineages possess superior storability under ambient conditions.

Rindyastuti and Siahaan (2019) investigated the impacts of natural ageing under ambient conditions. Their findings revealed that while seedling length remained statistically stable during the first month of storage 12.78 cm to 12.18 cm, a significant and gradual reduction was observed thereafter, reaching 10.64 cm at three months and dropping sharply to 7.77 cm after four months. Furthermore, seed viability declined from an initial 80% to 66.67% over a five-month period, accompanied by a reduction in seed moisture content from 12.39% to 10.45%. These results highlight the rapid physiological deterioration that occurs in Pterocarpus species when subjected to natural ageing.

Ouedraogo *et al*. (2025) investigated endangered African Rosewood (*Pterocarpus erinaceus*) establishes a critical quantitative framework for assessing seed storability through moisture sorption isotherms. Their results indicated that freshly harvested seeds, with germination percentages ranging from 96% to 98%. However, the study identified a specific "safe" moisture content (MC) threshold between 6% and 8% for maintaining long-term viability; seeds kept within this range exhibited no significant deterioration over a 12-month storage duration. In contrast, seeds stored at moisture levels exceeding 12% suffered a rapid decline in both vigor and viability within only 4 to 6 months. Furthermore, a clear trend of physiological ageing was documented as storage duration increased; specifically, the Mean Germination Time (MGT) rose from an initial 4 days in fresh seeds to 7.5 days after 9 months of storage. These findings suggest that for the genus *Pterocarpus*, monitoring metabolic speed (MGT) and maintaining moisture below the 9.5% EMC mark are vital strategies for mitigating the effects of natural ageing.

P t e r o c a r p u s m a r s u p i u m

Pradhan and Badola (2012) conducted a study on *Swertia chirayita* from the Sikkim Himalaya which suggests that thermal environments play a decisive role in maintaining seed viability and the speed of germination. Among the conditions tested which is ranging from ambient room temperature to sub-zero levels (-15°C). A consistent temperature of 4°C proved to be the most effective for long-term preservation. This specific refrigerated environment not only minimized the rate of decline in germination percentages but also ensured that the seeds required less time to sprout compared to those stored in more extreme or fluctuating temperatures.

Duong *et al* (2013) conducted research on *Jatropha curcas* seeds from diverse geographical origins confirms that their physiological stability is heavily influenced by the thermal environment. While seeds stored at a room temperature of 28°C lost all regenerative capacity within a year, those maintained in cooler settings remained viable. Interestingly, although the species exhibits orthodox storage behavior, extremely low temperatures (-23°C) proved less effective than a standard cool room at 4°C. The study suggests that the higher moisture content of the seeds (8–10%) likely caused internal damage during deep freezing storage, making 4°C the optimal thermal regime for maintaining health.

Aghilian *et al.* (2014) revealed that seeds respond differently to temperature

gradients; while some medicinal species-maintained stability in a refrigerated environment (4°C) while others exhibited distinct physiological shifts when kept at room temperature. These varying reactions highlight that the ideal storage condition is highly species-dependent, with thermal stability playing a critical role in either preserving or altering the initial germination capacity.

Tiwari and Das (2014) conducted an experiment on seven ethno-medicinal species in Chhattisgarh, India, which highlights that the efficacy of storage environments is highly dependent on the choice of packaging materials. The study demonstrated a significant interaction between thermal conditions and containment types; specifically, while polythene bags proved superior for maintaining seed viability at room temperature, aluminum foil emerged as the most effective medium when seeds were kept in low-temperature storage.

Lisboa *et al.* (2018) studied on *Thymus daenensis* Celak and revealed that while freezing at -20°C effectively preserves the original oil composition and ambient storage (25°C) can actually facilitate beneficial chemical conversions which increasing the concentrations of key compounds like thymol and carvacrol. Similarly, investigations into *Hyptis pectinata* L. confirmed that variations between freezer and room temperatures (32°C) lead to distinct fluctuations in the concentration of volatile constituents over time. These findings suggest that the chosen storage environment can be strategically used to either maintain a plant's initial state or potentially enhance specific therapeutic components through natural chemical reactions.

Amponsah *et al.* (2022) conducted a study on *Pterocarpus erinaceus*, an endangered species of arid and semi-arid regions of West Africa, evaluated the effects of pre-treatments and storage conditions on seed germination and viability. The results showed that extracted seeds had significantly higher germination compared to whole fruits, indicating the importance of seed extraction. Storage at low temperature (-10°C) minimized seed deterioration compared to ambient conditions. Moisture sorption studies revealed an S-shaped isotherm typical of orthodox seeds, best explained by the Henderson model. The study highlights the importance of proper seed extraction and controlled storage conditions for effective conservation of *Pterocarpus erinaceus*.

Tiwari *et al*. (2022) studied that maintaining the genetic integrity and regenerative potential of medicinal species requires a specialized approach to storage as germination success depends heavily on the interaction between packaging materials, temperature and storage duration. For instance, while low-temperature

environments (4°C) generally enhance the retention of total phenolics and flavonoids as compared to room temperature. The choice of container which is ranging from polythene bags to earthen pots is must be tailored to the specific biological requirements of each species. Ultimately, these findings suggest that implementing species-specific storage protocols is essential for the long-term conservation and sustainable management of forest-derived medicinal resources.

Pterocarpus marsupium

Pradhan and Badola (2012) conducted a study which utilized multivariate analysis to track physiological changes over a comprehensive 24-month period. While initial tests showed exceptionally high baseline germination rates of up to 100% and the passage of time naturally led to a gradual decrease in seed vigour. However, the robustness of the species was evident as a majority of the populations maintained over 50% germination capacity even after two years of storage. These longitudinal findings confirm that while time inevitably impacts seed quality, the rate of deterioration can be significantly managed through proper environmental control.

Duong *et al.* (2013) conducted a study over a comprehensive 24-month timeline for storage. The research tracked a significant and progressive decline in the seed's regenerative capacity. The data showed that germination remained stable during the first year in cold storage and substantial deterioration occurred between the 12-14 month marks. Despite this natural aging process, the study confirmed that more than half of the seeds remained viable after two years when kept at 4°C, proving that the species follows an orthodox storage pattern where the rate of decline can be managed but not entirely halted.

Aghilian *et al*. (2014) revealed influence of storage duration was equally diverse across the evaluated species over a 12-month period. While half of the species showed no significant change in viability and others experienced a notable increase in germination because of the natural breaking of dormancy or a steady decline as the storage period progressed. A small subset even displayed cyclic fluctuations in germination percentages. These findings, supported by tetrazolium (T_Z) testing and dormancy pre-treatments which suggest that the length of time a seed remains viable is governed by unique biological clocks that respond to the passage of months in storage.

Tiwari and Das (2014) studied the progression of seed quality over a nine-month duration which revealing that the timeline of storage significantly impacts regenerative potential regardless of the species. While initial observations showed that room-temperature storage initially supported higher germination percentages, these rates fluctuated based on the length of time the seeds remained in their respective environments. Because the response to storage duration varied even among seeds collected from different wild habitats. The findings suggest that conservation strategies must account for these temporal changes to ensure that medicinal plant resources remain viable for long-term restoration projects.

Bozoglu and Aybey (2024) revealed storage analysis of 4, 6, 8, 10 and 12 months in faba bean. The study demonstrated that the duration of storage significantly influences secondary growth parameters such as plumule and radicle length. The longest root measurements were observed in seeds stored for 8 months, while the maximum plumule length was recorded after a full 12 months of storage. These findings confirmed that faba beans experience minimal loss of viability during their first year of storage. It was noted that the standard 4 day counting window for germination rate may be too early for this species to show its full potential.

Pterocarpus mars

Eyong *et al*. (2011) conducted a study on selected medicinal plants including *Bryophyllum pinnatum, Ipomoea aquatica, Oldenlandia corymbosa, Ricinus communis, Terminalia bellirica, Tinospora cordifolia* and *Xanthium strumarium* to evaluate their phytochemical composition, total phenolic and flavonoid contents. Extraction was carried out using Soxhlet apparatus with different solvents, while standard methods were used for biochemical estimations. The results revealed the presence of key bioactive compounds such as proteins, carbohydrates, phenols, tannins, flavonoids and saponins in all plants. Quantitative analysis showed variation in phenolic and flavonoid contents among species, with higher levels observed in *Tinospora cordifolia* and *Terminalia bellirica*. The study highlights the therapeutic potential of these plants and supports their traditional medicinal use.

Kumar *et al.* (2011) investigated the physiological and biochemical transitions occurring in *Pongamia pinnata* seeds to determine the metabolic drivers of deterioration. The study specifically monitors the loss of membrane integrity and the

escalation of lipid peroxidation as indicators of cellular aging across varying thermal and humidity levels. Furthermore, it seeks to quantify the depletion of essential enzymatic activities, such as dehydrogenase and acid phosphatase, alongside shifts in soluble sugar and protein concentrations.

Laher *et al.* (2013) conducted study on evaluating the biochemical and phytochemical transformations in traditional medicinal plants during post-harvest storage to ensure therapeutic consistency. The study aims to quantify the degradation of secondary metabolites and antimicrobial potency across various plant species and tissue types under short-term aging. By comparing the pharmacological profiles of fresh and stored extracts, the objective is to determine how storage duration impacts antioxidant capacity and chemical concentration. These findings seek to establish standardized quality control parameters that safeguard the safety and efficacy of the medicinal plant industry.

Afzal *et al.* (2020) investigated the biochemical shifts in stored seeds. This study focuses on monitoring the degradation of essential macromolecules and membrane integrity under varying environmental conditions. The research revels fluctuations in total protein and oil concentrations to determine how moisture and temperature accelerate nutrient loss. Additionally, it measures lipid peroxidation through malondialdehyde levels and assesses cellular leakage via electrical conductivity to pinpoint the onset of physiological aging.

Paravar *et al.* (2023) conducted research to evaluate the physiological and biochemical transitions that occur in *Lallemantia iberica* seeds during prolonged storage. By monitoring fluctuations in antioxidant enzyme activities, such as catalase and ascorbate peroxidase, the study aims to quantify the internal defense mechanisms against oxidative stress. Furthermore, the research examines the degradation of membrane integrity and metabolic reserves, specifically focusing on amylase activity and soluble sugar depletion.

Farahani *et al.* (2025) identified the species-specific biochemical mechanisms that drive seed deterioration under aging conditions. By monitoring fluctuations in antioxidant enzyme activities, such as catalase and ascorbate peroxidase, the study seeks to quantify the internal defense responses of different plant varieties to thermal and moisture stress. Additionally, the objective is to assess the loss of membrane integrity by measuring increases in hydrogen peroxide, malondialdehyde and electrical conductivity. Ultimately, these measurements provide a comparative framework to

determine which species possess superior biochemical resilience during prolonged storage.

The experiment will be carried out in *Pterocarpus marsupim* Roxb. during 2026-2027 at department of seed science and technology, College of Agriculture, JAU, Junagadh. Seeds will be collected during March-July 2026 from well-established plantation at Botanical Garden, Paritalav, Junagadh Agricultural University, Junagadh. The experiment will be conducted to evaluate the effect of different harvesting time, storage conditions and storage duration on seed quality and biochemical parameters of Indian kino tree (*Pterocarpus marsupim* Roxb.).

A preliminary germination trial will be conducted in the Department of Seed Science and Technology to identify the most effective protocols for overcoming dormancy in *Pterocarpus marsupium* Roxb. This initial phase focuses on evaluating the impact of various pre-sowing treatments, including chemical scarification and organic treatment, on the overall germination percentage.

: Dept. of Seed Science and Technology,
CoA, Junagadh Agricultural University, Junagadh
: 2026-27
: Indian kino tree (*Pterocarpus marsupium* Roxb.)
: Completely Randomized Design (Factorial)
: 3 (Three)
:
: H_1 : February
H_2 : April
H_3 : June
: S_1 : Ambient
S_2 : Silica gel (low moisture storage)
S_3 : Refrigerator (4° C)
: D_1 : 0 month
D_2 : 3 month
D_3 : 6 month
D_4 : 9 month
D_5 : 12 month

T_1	$H_1S_1D_1$	T_{10}	$H_1S_2D_5$	T_{19}	$H_2S_1D_4$	T_{28}	$H_2S_3D_3$	T_{37}	$H_3S_2D_2$
T_2	$H_1S_1D_2$	T_{11}	$H_1S_3D_1$	T_{20}	$H_2S_1D_5$	T_{29}	$H_2S_3D_4$	T_{38}	$H_3S_2D_3$
T_3	$H_1S_1D_3$	T_{12}	$H_1S_3D_2$	T_{21}	$H_2S_2D_1$	T_{30}	$H_2S_3D_5$	T_{39}	$H_3S_2D_4$
T_4	$H_1S_1D_4$	T_{13}	$H_1S_3D_3$	T_{22}	$H_2S_2D_2$	T_{31}	$H_3S_1D_1$	T_{40}	$H_3S_2D_5$
T_5	$H_1S_1D_5$	T_{14}	$H_1S_3D_4$	T_{23}	$H_2S_2D_3$	T_{32}	$H_3S_1D_2$	T_{41}	$H_3S_3D_1$
T_6	$H_1S_2D_1$	T_{15}	$H_1S_3D_5$	T_{24}	$H_2S_2D_4$	T_{33}	$H_3S_1D_3$	T_{42}	$H_3S_3D_2$
T_7	$H_1S_2D_2$	T_{16}	$H_2S_1D_1$	T_{25}	$H_2S_2D_5$	T_{34}	$H_3S_1D_4$	T_{43}	$H_3S_3D_3$
T_8	$H_1S_2D_3$	T_{17}	$H_2S_1D_2$	T_{26}	$H_2S_3D_1$	T_{35}	$H_3S_1D_5$	T_{44}	$H_3S_3D_4$
T_9	$H_1S_2D_4$	T_{18}	$H_2S_1D_3$	T_{27}	$H_2S_3D_2$	T_{36}	$H_3S_2D_1$	T_{45}	$H_3S_3D_5$
Total no. of combinations = 3×3×5 = 45									

The number of days taken from the date of sowing to the appearance of the first germinated seed in each treatment will be recorded as days to initiation of germination.

The number of days taken from sowing to the final count of germination, when no further germination occurs, will be recorded. It reflects the total duration required for completion of germination as days to final germination.

Germination (%) will be calculated by counting the number of normal seedlings produced in each treatment and expressing it as a percentage of total seeds sown.

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It is measure of the average number of seeds germinating per day throughout the entire test period.

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Represents the maximum mean germination reached at any time during the test, serving as an indicator of the peak speed of the process. It is calculated by following formula given by Czabator 1962.

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Where,

G_1, G_2, G_3.....G_n, are cumulative germination percentage in the n^{th} time interval and T_1, T_2, T_3.....T_n are the time from the start of experiment to n^{th} interval.

It is composite objective value that integrates both the speed and the total completeness of the germination process. It will be calculated using following formula given by Czabator 1962.

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It is the average time required for a seed to germinate, which helps evaluate the speed and uniformity of the seed lot.

Mean germination time = $\frac{\text{B}}{}$

It is calculation that reflects the percentage of germination per day, where higher values indicate a faster and more vigorous germination rate.

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Where,

G_1, G_2, G_3.....G_n, are daily germination percentage in the n^{th} day and T_1, T_2, T_3.....T_n are the day at which germination was counted from the start of experiment to n^{th} interval.

The length of the primary root of normal seedlings will be measured in centimetres at the final count.

The length of the shoot from the base to the tip of the seedling will be measured in centimetres.

Total seedling length will be calculated by adding root length and shoot length. It provides an overall measure of seedling growth.

Fresh weight of seedlings will be recorded immediately after uprooting.

Seedlings will be dried in an oven and dry weight will be recorded.

Seedling vigour index will be calculated as per procedure prescribed by Abdul-Baki and Anderson (1973).

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Seedling vigour index will be calculated as per procedure prescribed by Abdul-Baki and Anderson (1973).

3 6طي) سلي ' Å Ò Í É Î Ñ Ô ÉÆ 3Ï Å Å Ä Ì Ø Ù Ç É ÇÇ È Ô

A 10 g sample was taken in a moisture container and weighed with its cover (M_1). The sample was evenly spread and weighed before drying (M_2). It was then dried in an oven at 103 ± 2°C for 17 ± 1 hours. After drying, the container was cooled in a desiccator for 30–45 minutes and weighed again (M_3).

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Where,

M_1 = Weight of empty container with cover

M_2 = Weight of container with sample before drying

M_3 = Weight of container with sample after drying

1.	Peroxidase (POX)	Yu *et. al.* (2003)
2.	Catalase (CAT)	Hadwan and Abed *et. al*. (2016)
3.	Tetrazolium test	Moore (1973)
4.	Estimation true protein	Lowry *et al*. (1951)
5.	Estimation of total soluble sugars	Dubois *et al*. (1956)
6.	Determination of α-amylase activity	Shuster and Gifford (1962)
7.	H_2O_2 determination	Mukharjee and Choudhuri (1983)
8.	Electrical conductivity	Kocheva *et al*. (2005)
9.	Membrane stability index (MSI)	Sairam (1994)

The experiment data collected from experiment will be analysed statically by adopting Completely Randomized Design (Factorial) as described by Gomez and Gomez (1984).

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